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Research Article

PHARMACOGNOSTIC STUDIES OF *AESCHYNOMENE INDICA* L.

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ABSTRACT

Aeschynomene indica is a swampy medicinal plant used to treat kidney stones and urinary disorders by the Chenchu tribes and the local herbalists. All plant parts Leaf, Flower and Fruits were screened out for their secondary metabolites in the four selected solvents and observed the presence of alkaloids, flavonoids, phenols, steroids and glycosides. Qualitative analysis of flavonoids (6 identified and 1 unidentified) phenols (19 identified and 5 unidentified) anthocyanidins (3) was carried out. Isochlorogenic acid, coumarin, p-hydroxy benzoic acid as the major phenolic compounds; kaempferol and apigenin as major flavonoids; delphinidin, malvidin and peonidin as the major anthocynadins. Antimicrobial activity of the leaf cold water, hot water, methanol and alcohol extracts are more efficient on the selected four pathogens of gram positive (*Bacillus subtilis, Staphylococcus aureus*) and gram negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria than the control drugs *Ampicillin* and *Gentamycin* with an average inhibitory zone of 20 mm to 41 mm at 10 mg/well concentration of the drug. Minimum Inhibitory Concentrations were observed between 0.55-0.75 mg/ml equal to that of the control drugs.

Keywords: *Aeschynomene indica*, Chenchu tribes, Secondary metabolites, Qualitative analysis, Minimum Inhibitory Concentration.

INTRODUCTION

Aeschynomene indica belongs to the family Fabaceae is a sub shrub in swampy areas. It is commonly known as Tella-Jeeluga, (Telugu), Didhen, Phulan (Hindi), Netti, Attunetti (Tamil). Stem nodules are present on the basal stems which are large in size than the root nodules and also rooting from the basal nodes. It is used as spermicidal[1] and also as Charcoal for gun powder[2]. This species is used in the category of genital stimulant or depressant poisonous or repellent, fodder, composting, manuring, farming, hunting and fishing apparatus, household, domestic and personal items[3]. Oraon tribes use the root as magical cure for Jaundice[4]. In Ayurveda "Charak samhita" it is known as Ashanibhed (*A.indica*) is recommended for biliary calculi. The decoction of Kusa, Ashibhed, Patala, Trikanthaka, Sirisha, Punarnava and Shilajit is given against Pittaji Ashmari[5]. In Siddha this species called as kidaichi (sadai poondu) where leaves are used for treating leprosy. *A. indica* finds an important place in Bengal small scale industry where quite a good number of art forms are prepared and also exported. Locally known as sola, utilized for decorating the idols of Goddesses Durga Kali. Sola items like mukut are worn by both bride and the groom in Bengal[6]. Kani tribes of Tirunelveli hills of Tamilnadu used *A.indica* (Kodithuvarai) as antidote against snake bite for 40 days orally along with leaves of *Andrographis paniculata* and *A.lineata,* roots of *Thespesia populnea* and stem bark of *Strychnous nux vomica*. The decotion is also mixed with bathing water to treat snake bite[7]. Chenchu tribes of Nallamalai hills used this plant for curing kidney troubles and the leaves are edible[8]. It is also known as 'Pilliya' by the tribe Gujjars of Uttar Pradesh, used against cattle skin eczema the external application of plant paste along with emblica fruit paste [9].

MATERIALS AND METHODS

Collection of Plant material

The species *A.indica* was collected from Mallemadugu dam along the water hedges near dodlamitta area of Renigunta Mandal. The taxonomical identity of the plant was determined and authenticated from the literature available in the Department of Botany and the voucher specimen (CA.27) was deposited in the Department Herbarium. The present work was carried out in the Department of Botany, S.V.University, Tirupati. Leaf, flowers and fruit material was thoroughly washed and dried under shade at 28 ± 2°C for about 10 days. The dried materials were ground well into fine powders in a mixer grinder and sieved to give particle size of 50-150mm. The powders were stored in air sealed polythene bags at room temperature until further use.

Phytochemical Analysis

Solubility

The solubility of different powder extracts was studied in eight solvents as Hot water, Cold water, Benzene, Hexane, Ethyl acetate, Chloroform, Methanol and Ethanol based on polarity gradience.

Preliminary Phytochemical analysis of secondary metabolites

All the extracts were subjected to preliminary Phytochemical screening for the Presence or absence of various secondary metabolites such as alkaloids, flavonoids, phenols, Terpinoids, steroids, Anthocynadins, anthroquinones, saponins, tannins, lignins, Indoles and glycosides by the standard methods[10].

Extractive value determination

Coarsely powdered air-dried material 20 g was placed in a glass stopper conical flask and macerated with 200 ml of water shaking frequently, and then allowing it to stand for 18 hours. Filter it rapidly through Whatmann No. 1 filter paper, taking care not to lose any solvent. Transfer 25 ml filtrate to a flat bottom dish and evaporate it on a water bath. Dry at 105°C for 6 hours, cool in a desiccators for 30 minutes and weigh it immediately. Calculate the content of extractable matter in% of air-dried material by the standard method[11].

Physico-Chemical Analysis

The total ash is particularly important in the evaluation of the purity of the drug. For the determination of ash values, the leaf, flowers and fruits powder is screened for the following tests [12-15].

Total ash

About 3 g of each powder is accurately weighed and taken separately in silica crucible, which was previously ignited and weighed. The powder was spread as a fine layer on the bottom of crucible. The powder was incinerated gradually by increasing temperature to make it dull red hot until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash was calculated with reference to the air-dried powder.

Acid insoluble ash

The ash obtained as described above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a crucible, ignited and weighed. The procedure was

repeated to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air -dried drug.

Water soluble ash

The ash obtained as described for the total ash, was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred into silica crucible, ignited for 15 min. and weighed. The procedure was repeated to get a constant weight. The weight of insoluble matter was subtracted from the weight of total ash. The difference of weight was considered as water soluble ash. The percentage of water-soluble ash was calculated with reference to air-dried parts respectively.

Sulfated ash

A silica crucible was heated to red for 10 min. and was allowed to cool in desiccators and weighed. One gram of substance was accurately weighed and transferred to the crucible. It was ignited gently at first, until the substance was thoroughly charred. Then the residue was cooled and moistened with 1 ml of concentrated sulfuric acid, heated gently until white fumes are no longer evolved and ignited at 800°C ± 25°C until all black particles have disappeared. The ignition was conducted in a place protected from air currents. The crucible was allowed to cool. A few drops of concentrated sulfuric acid was added and heated. Ignited as before and was allowed to cool and weighed. The percentage of sulphated ash was calculated with reference to air-dried parts respectively.

Swelling index

It is defined as the volume in milliliters occupied by 1g of a drug. The drug is treated with 1.0 ml ethanol (96%) and 25 ml water in a graduated cylinder, shaken every 10 minutes for 1 h and allowed to stand. The drugs have mucilage (has a property to swell after absorbing plenty of water) as a Phyto-constituent may have different swelling index and therefore, provide the useful information.

Foaming index

The drug containing saponins has the capability to form froth which depends upon the nature of drug and/or quantity of saponins present. This parameter also provides useful information and help in quality control of the drug.

Moisture content

Air dried material of 10g was dried in an oven at 105°C. The loss of weight was calculated and values were tabulated.

Foreign matter

The term "Foreign Matter" is used to designate any matter, which does not form a part of the drug. 100g of the powdered drug is taken and spread out in a thin layer. Plant material collected should be free from foreign matters like soil, insect parts or animal excreta.

Fluorescence Analysis

A small quantity of dried and finely powdered plant parts like Leaf, flowers and fruits were placed on a grease free clean microscopic slide and added 1-2 drops of freshly prepared reagent solutions mixed gently tilting the slide and waited for 1-2 min. Then the slide was placed inside the Ultra Violet viewer chamber and viewed in Day light, Short (245nm) and long (360nm) Ultra Violet radiation .The colors observed by application of different reagents in different radiations were recorded [11].

Qualitative Analysis of Phenolic Compounds

Chemicals, Glassware & Equipment

Peroxide free Diethyl ether, Anhydrous Na2Co3, HCl, Ethanol, Benzene, Acetic Acid, Sodium Formate, Formic Acid, Sulphanilic Acid, Sodium Nitrite, Sodium Carbonate, Para Nitriline, Ferric Chloride, Separatory funnel, Beakers, Conical flasks, Measuring Jars, Capillary tube, Test tubes, Glass tanks, Petri plates and Automiser.

Extraction of Phenolic compounds

Fresh leaves, flowers and fruits were collected and extracted by using the method.[16] About 30 gm of the healthy and fresh, leaves, flowers and fruits were macerated in approximate 100 ml of 2N HCl. The homogenate was digested on a boiling water bath for about 30min. The contents were cooled and filtered through Whatmann no: 1 filter paper. The filtrate was extracted with Peroxide free diethyl ether (solvent ether) repeatedly. The cooled extract was concentrated to a small volume and was treated thrice with 25ml of 5% anhydrous Na_2Co_3 solution. The cooled Na_2Co_3 solution was adjusted to P^H 2 with concentrated HCl. The acidified fraction was then extracted with equal volumes of fresh diethyl ether for three times and the combined ether extracts were washed with 2ml of distilled water for 3times to remove the traces of HCl. The ether soluble water was removed by freezing the extract and then the ether was evaporated to dryness on water bath at 98°C. The resulting residue was dissolved in 1ml of 95% ethanol and was preserved at low temperature in a dark container until used.

Separation of Phenolic compounds

1gm of the extract was spotted on 23 × 29cm Whatmann No: 1 chromatographic paper with the help of a micropipette. The origin of the spot was dried immediately with the help of hair drier. The dried sheets were run in bi-dimensional ascending chromatography by using rectangular chromatographic glass tank. The Chambers are saturated with the chromatographic solvents one day before. The development of Chromatograms has to be carried out at 22°-24°C.

Solvent I: Benzene: Acetic acid: water (60: 70: 30) v/v/v (upper layer of this mixture used for first direction).

Solvent II: Sodium formate: Formic acid: water (10: 1: 200) **v/v/v** (used for second direction).

The paper after development was removed from the tank and dried at room temperature. The dried sheet was examined under UV light and the fluorescent regions were marked. Then the paper was exposed to ammonia vapors were also observed under UV light and the new fluorescent spots were also marked.

Identification of Phenolic compounds using chromogenic spraying reagents

To identify the Phenolic compounds separated on the chromatograms were sprayed with Diazotized Sulphanilic acid, Paranitranilins and ferric chloride reagent with the help of an automizer. The Phenolic compounds were identified by calculating their Rf values of individual spot colors with chromogenic sprays and finally confirmed with authentic samples by co-chromatography.

Preparation of Chromogenic sprays reagents

Diazotized Sulphanilic acid: Sulphanilic acid Solution, 5% Sodium Nitrite Solution: 20% Anhydrous Sodium Carbonate solution, Diazotized Para Nitraniline Reagent, 5%Anhydrous Sodium Nitrite solution, 10% Sodium Carbonate solution, Ferric Chloride reagent.

Qualitative Analysis of Flavonoid Compounds

Chemicals &Glassware

Methanol, Chloroform, Alcohol 95%, Isopropyl alcohol, Ammonia, Sulphanilic acid, Concentrated HCl, Sodium Nitrate, Sodium Carbonate, Separatory funnel, Beakers, Conical flasks, Measuring jar, Capillary tube, Test tubes, Glass tanks, Petri plates, Atomizer.

Extraction of flavonoids compounds

The flavonoid compounds were extracted according to the standard method[10]. About 2g of dried leaf, flower and fruit powder was taken in a boiling test tube separately heated up to 40°C and then 18ml of methanol and 2ml of water (9:1) was added. Shaken well and was kept for about 24 hrs at room temperature. After that the upper clear solution of the extract was transferred to another test tube. To the remaining residue in the test tube, again 5ml of methanol and 5ml of water (1:1) was added. Stirred well and the contents were

kept for 24 hours and the clear extract thus obtained was pooled up with the earlier sample. The combined extract was mixed well and filtered through the cotton. The filtrate was evaporated to about 1/3 of the original volume and the resultant aqueous extract was taken into a separatory funnel and then extracted with 10ml of chloroform. This process was repeated 2or 3 times. All the chloroform extracts were combined and evaporated to dryness under vaccume in a rotatory evaporator and the dried residue was dissolved in 1ml of 95% alcohol which was stored at low temperature in the dark until used.

Separations of flavonoid compounds

1 gm of the extract was spotted on 23x29cm Whatmann No: 1 chromatographic filter paper with the help of a micropipette. The origin of the spot area was dried immediately with the help of hair drier. The dried sheets were run in unidimensional ascending chromatography by using rectangular chromatographic glass tanks which can accommodate 4 sheets at a time. The chromatographic chambers were saturated with any of the following solvents one day before the development of the chromatograms at 22-24°C.

1. Isopropyl alcohol: Ammonia (25%): Water (8: 1: 1) v/v/v.

2. n-Butanol: Acetic acid: Water (4: 1: 5) v/v/v (top layer was used).

3. Conc.HCl: Acetic acid: water (3: 30: 10) v/v/v.

4. Phenol: Water (3: 1) v/v.

The chromatograms after unidimensional development were removed from the tanks and dried at room temperature. The dried sheet was observed under UV light and the fluorescent regions were marked. The paper while exposed to ammonia was also observed under UV light and the new fluorescent spots also marked.

Identification of flavonoids using chromogenic spray reagents

To identify the flavonoid compounds separated on the chromatograms were sprayed with chromogenic spray reagents with the help of an atomizer. The flavonoid compounds were identified by calculating their Rf values of individual compound along with authentic samples by co-chromatographic studies.

Preparation of Chromogenic spray reagents

Sulphanilic acid solution, Sodium Nitrate solution, Anhydrous sodium carbonate solution.

Qualitative Analysis of Anthocyanidins Compounds

Chemicals & Glassware

2N HCl, Ethyl acetate, Iso-amyl alcohol, Methanolic HCl, Beakers, Separatory funnel, Conical flasks, Measuring Jar, Capillary tube, Test Tubes, Glass tanks, Petri plates, Automiser, Dark container.

Extraction for Anthocyanidins Compounds

Anthocyanidins were extracted following the method[10]. About 5 gms of flowers were heated in 20ml of 2N HCl in a boiling test tube for 40 minutes at 100ºC. The extract was cooled and decanted from the plant tissue. The extract was twice washed with 20ml of ethyl acetate to remove flavonoid compounds. The ethyl acetate layers discarded and the remaining aqueous extract heated at 80º C for 30mts to remove the last traces of ethyl acetate. The pigment was then extracted into a small volume of Iso-amyl alcohol in a separatory funnel. The Iso-amyl alcohol extract was evaporated to dryness on a boiling water bath. The Anthocyanidins in the residue was eluded with 1ml of 1% methanolic HCl and was preserved at low temperature in a dark container until used.

Separation of Anthocyanidins Compounds

1 gm of the extract of Anthocyanidins was spotted on 23x29 cm whatmann No: 1 chromatographic filter paper with the help of micropipette. Origin of the spot area was dried immediately with the help of hair drier. The dried sheet was run in uni dimensional ascending chromatography by using rectangular chromatographic glass tanks. The chromatographic chambers were saturated with an appropriate quantity of suitable solvent one day before the development of the chromatogram at 22-24ºC

1. Conc. HCl : Formic acid :Water (2 :5 : 3) v/v/v
2. n-Butanol : Acetic acid : Water (4 : 1 : 5) v/v/v
3. n- Butanol : 2N HCl (1 : !) v/v/v

The chromatograms after unidimensional development were taken from the glass chambers and dried at room temperature. The dried papers were observed under UV light and the fluorescent regions were marked. The sheets were also observed under UV Light while exposing to ammonia vapors and fluorescent intensified spots were recorded. The Rf values and colours of the spots were determined.

Antibacterial Activity

Extracts preparation

Dried leaf powder (25g) was packed in a Whatmann no.1 filter paper and was extracted in a Soxlet apparatus using 100ml of each (Alcohol, Methanol, Hot water and Cold water) extracts were dried and stored in a refrigerator at 4°C.

Bacterial cultures

The bacterial cultures *Bacillus subtilis* (MTCC-441) causes Pneumonia, diarrhoea. *Staphylococcus aureus* (MTCC-737) causes bone and joint pains, skin infections, boils; *Escherichia coli* (MTCC-443) causes urinary tract infections, Pneumonia, *Pseudomonas aeruginosa* (MTCC-741) causes urinary infections and Pneumonia. The above strains were procured from the Department of Microbiology S.V. University Tirupati and also from the SVIMS Tirupati.

Preparation of inoculums

Stock cultures were maintained at 4°C of nutrient agar slants. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient agar medium and were incubated without agitation for 24hrs at 37°C.

Preparation of the medium

To prepare 1lit of nutrient agar medium 3g of beef extract, 3g of peptone, 15g of agar was used. The ingredients were accurately weighed using digital electronic balance and dissolved in one liter of distilled water before the addition of agar; the P^H of the medium was adjusted to 7.0 by adding few drops of 0.1N NaoH/HCl using digital P^H meter. Later this medium was transferred to conical flasks and plugged with nonabsorbent cotton. Medium was then sterilized by autoclaving at 15lbs for 20min cooled to 4 °C and used for the study.

Agar well diffusion assay

The antibacterial activity of the leaf extracts was determined using agar well diffusion method[17]with slight modifications. Nutrient agar was inoculated with the selected microorganisms by spreading the bacterial inoculums on the media. Wells (8 mm diameter) were punched in the agar and filled with 20µl plant extracts. Control wells containing pure solvents (negative control) or standard antibiotic (positive control) viz., *Gentamycin* (30mg) and *Amphicilin* (10 mg). The plates were incubated at 37°C for 18hrs. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition for the respective drug. The relative antibacterial potency was calculated by comparing its zone of inhibition with that of the standard drug. The data of crude drug activity is given in the mean of quadruplicates along with the standard error.

Minimum Inhibitory Concentration

MIC is defined as the lowest concentration where no visible turbidity is observed in the test tube (bacterial concentration).The method[18] modified by Usman[19] was employed. In this method the broth dilution technique was used, where the leaf extract was prepared to the highest concentration of 10mg/ml (stock concentration). By adding sterile distilled water and serially diluted (two fold dilution)

using the nutritive broth and later inoculated with 0.2ml standardized suspension of the test organisms. After 18hrs of incubation at 37°C., the test tubes were observed for turbidity. The lowest concentration of the tube that did not show any visible growth can be considered as the MIC.

RESULTS

Solubility

Solubility with eight solvents of leaf powder revealed in increased gradience from hot water to alcohol (Hot water> Cold water> Benzene> Hexane> Ethyl acetate> Chloroform> Methanol> Ethanol). It is highest in Hot Water and less in Ethanol respectively**.**

Preliminary phytochemical screening- secondary metabolites (Table-1)

Water Solvents are rich in containing more number of bioactive compounds in all parts (Leaves, Flowers and Fruits). The main constituents are alkaloids, flavonoids, phenols, saponins and tannins. Whereas, terpinoids and lignins are present only in the leaves; steroids in leaves and flowers; glycosides in leaves and flowers; indoles in flowers only.

Extractive values: (Table-2)

The values indicate the percentage of the constituents present in the crude drug. The leaf extractive value are high in cold water, hot water, methanol than flowers and fruits, followed by hot water, alcohol, methanol and chloroform. In fruits alcoholic and chloroform extracts are more than leaf and flowers. In all parts of the plant cold water extracts are more when compared to other solvent extracts.

Physico – chemical analysis: (Table-3)

The total ash value, Water soluble ash and Sulphated ash of the fruit are high when compared to leaf and flower. The Acid insoluble ash and foaming index content is high in leaf and Flower compared to the fruit. In the present investigation considerable amount of the ash values were noticed in leaf, flower and fruit. These findings can be employed as purity parameters for the evaluation of *A.indica* drug for any adulteration.

Table 1: Preliminary Phytochemical Screening of Secondary Metabolites

S. No.	Name of the Test	Leaf				Flower				Fruit			
		CW	HW	AE	ME	CW	HW	AE	ME	CW	HW	AE	ME
1	**Alkaloids**												
	Mayers test	-	-	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
	Wagner's test	+ +	+ +	+ +	+ +	+ +	+ +	-	+ +	+ +	+ +	+ +	+ +
2	**Flavonoids**												
	Shinodons test	+ +	+ +	-	-	-	-	-	-	-	-	-	-
	Fecl3 test	+ +	+ +	-	-	+ +	+ +	+ +	+ +	+ +	+ +	+ + +	+ +
3	**Phenols**												
	Fecl3 test	+ +	+ +	+ + +	+ + +	+ +	+ +	-	-	+ +	+ +	+ + +	+ +
	Ellagicacid test	-	-	-	-	-	-	-	-	-	-	-	-
4	**Steroids-**												
	Salkowski test	+ +	+ +	-	+ +	-	-	-	-	-	+ +	+ +	+ +
	Liebermann's Burchard test	+ +	+ +	+ +	+ +	-	-	-	-	-	-	+ +	+ +
5	**Terpinoids**												
	Liebermann's Burchard test	+ +	+ +	-	+ +	-	-	-	-	-	-	-	-
6	**Saponins**	+ +	+ +	-	-	+ +	+ +	-	-	+ +	+ +	-	-
7	**Anthocynadins**	-	-	+ +	+ +	-	-	+ +	+ +	-	-	-	-
8	**Tannins**												
	Gelatin test	+ +	+ +	-	-	+ +	+ +	-	-	-	-	-	-
	Fecl3 test	+ +	+ +	-	-	+ +	+ +	-	-	+ +	+ +	+ +	+ +
9	**Lignins**	-	-	-	+ +	-	-	-	-	-	-	-	-
10	**Indols**												
	Enrilich test	-	-	-	-	-	-	-	-	-	-	-	+ +
11	**Glycosides**												
	Keller-kilani test	-	-	+ +	-	+ +	+ +	+ +	+ +	-	-	-	-

+ =indicates the presence of Secondary Metabolites. - =Indicates the absences of Secondary Metabolites. **CW** = Cold Water Extract; **HW** = Hot Water Extract; **AE** = Alcoholic Extract; **ME** = Methanolic Extract.

Table 2: Extractive Values

S. No.	Treatment	Leaf	Flower	Fruit
		% of extraction	% of extraction	% of extraction
1	Cold water	14.4	13.6	11.6
2	Hot water	12.0	11.6	10.6
3	Alcohol	8.4	8.4	9.4
4	Methanol	8.48	7.2	7.2
5	Chloroform	6.4	6.4	6.9
6	Benzene	2.4	2.4	2.4
7	Hexane	1.6	1.6	0.8
8	Ethyl acetate	1.6	0.8	1.2

Table 3: Physico Chemical Parameters

S. No.	Parameters	Leaf	Flower	Fruit
1	Total ash	6.0 % w /w	5.8% w /w	6.8% w /w
2	Acid insoluble ash	0.6% w /w	0.6% w /w	0.4% w /w
3	Water insoluble ash	3.9% w /w	3.6% w /w	4.0% w /w
4	Sulphated ash	2.6% w /w	2.8% w /w	3.2% w /w
5	Foreign matter	0.0% w /w	0.0% w /w	0.0% w /w
6	Swelling index	0.0	0.0	0.0
7	Foaming index	5.0	5.0	1.0
8	Moisture content	6.2% w /w	7.1% w /w	4.1% w /w

Fluorescence analysis: (Table-4)

Powdered drug under the ultra violet and ordinary light when treated with different reagents emitted various color radiations which help in identifying the drug in the powdered drug for the adulteration and substitution by any other drug. This plays a crucial role in standardization of crude drug. The main colors represents by all parts ranges from light yellow, reddish yellow (in day light) light green, dark green, brownish green and blackish green (250-270 nm) light green, blackish green (360-390nm).

Table 4: Fluorescence Analysis

S. No.	Chemical Analysis	Leaf			Flower			Fruit		
		Day light	250-270	360-390	Day light	250-270	360-390	Day light	250-270	360-390
1	Powder as Such	Y	L.G	G	W.Y	L.Y	Y	L.G	L.G	G
2	50% H_2SO_4	R.Y	Y.G	L.G	L.Y	Y	W	G	G	L.G
3	Conc. HCl	R.B	B	B.G	B	G.B	B	B	B	G.B
4	50% HCl	R.G	B	B	G	G	B	B.G	B	B
5	50% HNO3	L.O	Y.G	B.G	O	G	B	G	B.G	B
6	Ethanol	L.G	Y.G	G	Y	Y	R.Y	T.G	G	B
7	NaOH + Water	M.G	L.G	B	W.Y	W	L.Y	G	G.Y	L.G
8	10% NaOH	B	L.B	Br	Br	L.Y	W	B.G	B	B
9	Conc. H_2SO_4	Y	Y.G	G	Y	B.Y	L.Y	B	B.G	B
10	Conc. HNO_3	G.Y	Y.G	G	R.Y	G.Y	Y	O	G.Y	L.G
11	5% $FeCl_3$	G	B.G	B	B	G	G.B	B	B	B
12	5% KOH	B.R	B.G	B	Y	L.G	B	B.G	G	B.G
13	With water	G	L.G	L.G	R.Y	L.Y	L.Y	G	L.G	G
14	CH_3COOOH	L.G	Br.G	BR.G	Y	Y	L.Y	G	G	B
15	1N HCl	L.P	L.G	G	W.Y	Y	W.Y	M.G	G	G.G

B-Black **B.R-**Blackish Red **L.O-**Light Orange **R.Y-** Reddish Yellow, **Br-**Brown **B.Y-**Blackish Yellow **L.P-**Light Pink **W.Y-** Whitish Yellow, **G-**Green **Br.G-** Brownish Green **L.Y-** Light Yellow **Y.G-** Yellowish Green, **O-**Orange **G.B-** Greenish Black **M.G-** Muddy Green, **W-**White **G.G-** Golden Green **R.B –** Reddish Black, **Y-** Yellow **L.G-** Light Green **R.G -**Reddish Green

Qualitative Analysis of Phenols (Table-5a, 5b, 5c) (Plate-1)

Nearly 12 Phenolic compounds were observed in the leaves of *A.indica* out of which 2 are un identified. Mainly Iso-Chlorogenic acid is more in quality with buff colour, and Coumarin in more quantity spreading in larger area with Yellow colour. Other compounds are Neo-Chlorogenic acid, Phloroglucinol, β- Resorcyclic acid, Trans-p-Coumaric acid, Scopoletin, Aesculetin and Cinnamic acid. In flowers there are 2 unidentified and 12 identified Phenolic compounds. In this the Iso-Chlorogenic acid is more in brown color and P-hydroxy benzoic acid is having more quantity with yellow color. In fruits there are 11 identified and one unidentified Phenolic compounds. In this Iso-Chlorogenic acid is more in quality and Phloretic acid is having more quantity.

Table 5a: Phenols in leaf

S. No.	Rf values in solvent		UV fluorescence		Colour with spray reagents			Identified Compound
	I	II	With NH_3	Without NH_3	Sulphanilic acid	Para Nitriline	Ferric Chloride	
1&2	0.04	0.19	Faint blue	Duck egg green	Buff	Brown	Green	Iso-Chlorogenic acid*
3	0.04	0.55	None	None	Buff	Brown	Dark green	Phloroglucinol
4	0.04	0.86	Duck egg green	Bright duckegg green	Brown	Orange purple	Green	Neo-chlorogenic acid
5	0.28	0.47	White blue	Bright blue	Brown	Grey	None	Aesculetin
6	0.26	0.77	None	None	Dark brown	Brown	Reddish purple	β-Resorcyclic acid
7	0.50	0.38	Light blue	Deep blue	Light brown	Blue	None	Trans-p-Coumaric acid
8	0.11	0.02	None	None	Yellow	Blue	Green	Un Identified Compound I
9	0.28	0.11	None	None	Bright brown	Purple	None	Un Identified Compound II
10	0.35	0.27	Bright blue	Very bright blue	Yellow	Brown	None	Scopoletin
11	0.95	0.58	None	None	Yellow	Violet	None	Coumarin**
12	0.92	0.03	None	None	Yellow	Green	None	Cinnamic acid

Table 5b: Phenols in Flowers

S. No.	Rf values in solvent		UV fluorescence		Colour with spray reagents			Identified Compound
	I	II	With NH_3	Without NH_3	Sulphanilic acid	Para nitriline	Ferric chloride	
1&2	0.04	0.18	Faint blue	Duck Egg green	Light brown	Brown	Brown	Iso-chlorogenic acid*
3	0.02	0.43	None	None	Bright brown	Brown	Dark green	Phloroglucinol
4	0.10	0.37	Blue	Blue	Buff	Light brown	Dark green	Caffeic acid
5	0.08	0.56	Bright blue	Yellow	Light pink	Blue	Violet blue	Gentisic acid
6	0.08	0.77	None	None	Yellow	Brown	None	Un Identified Compound I
7	0.13	0.95	None	None	Pink	Brown	Purple	α-Resorcyclic acid
8	0.71	0.92	None	None	Pink	Purple	Light brown	Vanillic acid
9	0.39	0.69	None	None	Yellow	Light pink	None	p-hydroxy Benzoic acid**
10	0.67	0.58	Yellow	Yellow green	Light yellow	Blue	None	O-Coumaric acid
11	0.69	0.38	Green	Bright green	Pale pink	Bright green	None	Trans-Sinapic acid
12	0.43	0.03	Dark blue	Dark blue	Yellow	Pink	Light brown	Unidentified Compound II
13	0.93	0.23	Dark blue	Dark blue	Yellow	Pink	Light brown	Salicylic acid
14	0.96	0.69	None	None	Yellow	Violet	None	Coumarin

Table 5c: Phenols in Fruits

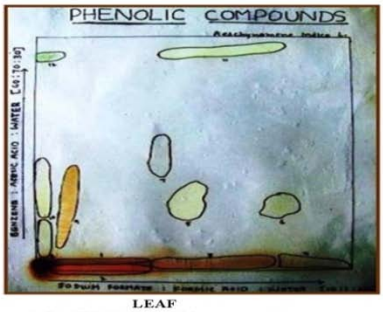
S. No.	Rf values in solvent		UV fluorescence		Colour with spray reagents			Identified Compound
	I	II	With NH_3	Without NH_3	Sulphanilic acid	Para nitriline	Ferric chloride	
1	0.05	0.21	Faint blue	Duck egg green	**Buff**	Brown	Green	**Iso-chlorogenic acid***
2	0.02	0.47	None	None	Light pink	Brown	Dark green	Phloroglucinol
3	0.13	0.34	Blue	Blue	Buff	Light brown	Dark green	Caffeic acid
4	0.11	0.58	Bright blue	Yellow	Buff	Blue	Violet blue	Gentisic acid
5	0.28	0.63	None	None	Brown	Brown	Reddish purple	β-Resorcyclic acid
6	0.16	0.95	None	None	Brown	Orange brown	Light brown	α-Resorcyclic acid
7	0.59	0.66	None	None	**Yellow**	Purple white	None	**Phloretic acid****
8	0.52	0.47	Light blue	Deep blue	Light yellow	Blue	None	Trans-p-Coumaric acid
9	0.72	0.34	Green	Bright green	Pink	Bright green	None	Trans Sinapic acid
10	0.93	0.28	Dark blue	Blue	Light orange	Pink	Light brown	Salicylic acid
11	0.95	0.71	None	None	Yellow	Violet	None	Coumarin
12	0.50	0.02	Green	Bright green	Light yellow	Bright green	None	Unidentified Compound I

Solvent I: Benzene: Acetic Acid: Water (60:70:30) v/v/v; Solvent II: Sodium Formate: Formic Acid: Water (10:1:200) v/v/v

* Compound showing more Color **Compound showing more Quantity

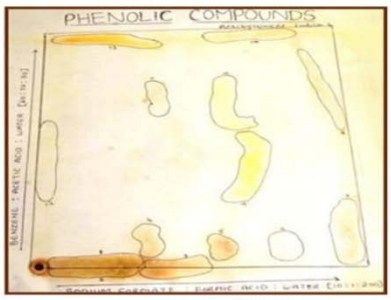
PLATE 1

Phenols



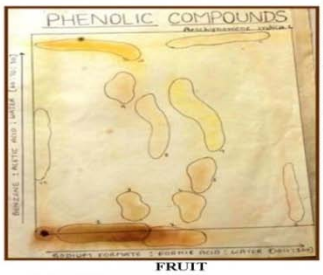
LEAF

1. Iso-Chlorogenic Acid
2. Iso-Chlorogenic Acid
3. Phloroglucinal
4. Neo-Chlorogenic Acid
5. Aesculetin
6. β-Resorcyclic Acid
7. Trans-P-Coumaric Acid
8. Unidentified compound I
9. Unidentified compound II
10. Scopoletin
11. Coumarin
12. Cinnamic Acid



Flowers

1. Iso-Chlorogenic Acid
2. Iso-Chlorogenic Acid
3. Phloroglucinal
4. Caffeic Acid
5. Gentisic Acid
6. Unidentified compound I
7. α- Resorcyclic Acid
8. Vanillic Acid
9. P-Hydroxy Benzoic Acid
10. O-Coumaric Acid
11. Trans-Sinapic Acid
12. Unidentified compound II
13. Salicylic Acid
14. Coumarin



FRUIT

1. Iso-Chlorogenic Acid
2. Phloroglucinal
3. m-Hydroxy Benzoic Acid
4. Gentisic Acid
5. β-Resorcyclic Acid
6. α- Resorcyclic Acid
7. Phloretic Acid
8. Trans-P-Coumaric Acid
9. Trans-Sinapic Acid
10. Salicylic Acid
11. Coumarin
12. Unidentified compound I

Qualitative Analysis of Flavonoids (Tables-6a, 6b, 6c) (Plate-2)

Leaves contain three identified and one unidentified compound as Myricetin (pink), Apigenin (Brown), Kaempferol (Orange) and unidentified (Yellow). Flowers contain Kaempferol (Orange) and Apigenin (Light Brown).In fruits Myricetin (Pink), Kaemferol (Light Yellow) and Orientin (Greenish Yellow). Kaempferol is the common and major compound in all parts.

Table 6a: Flavonoids in Leaf

S. No.	Rf Values In Solvent	UV Flourescence		Colour with spray reagents			Identified Compound
		Without NH_3	With NH_3	Sulphanilic Acid	1% Alcoholic $FeCl_3$	1% $AlCl_3$	
1	0.08	Yellow	Bright yellow	Pink	Olive	Green yellow	Myricetin
2	0.18	Green	Light yellow	Yellow	Pale yellow	None	Un Identified Compound I
3	0.3	Green yellow	Bright yellow	Orange	Green yellow	Yellow	Kaempferol*
4	0.62	Reddish brown	Reddish brown	Light brown	Pale yellow	None	Apigenin**

Table 6b: Flavonoids in Flowers

1	0.31	Green Yellow	Bright Yellow	Orange Brown	Greenish Yellow	Yellow	Kaempferol***
2	0.70	Yellow	Yellow Green	Light Brown	None	None	Orientin

Table 6c: Flavonoids in Fruits

1	0.07	Yellow	Bright Yellow	Pink	Olive	Green Yellow	Myricetin
2	0.35	Green Yellow	Bright Yellow	Light Yellow	Green Yellow	Yellow	Kaempferol***
3	0.75	Yellow	Yellow Green	Greenish Yellow	None	None	Orientin

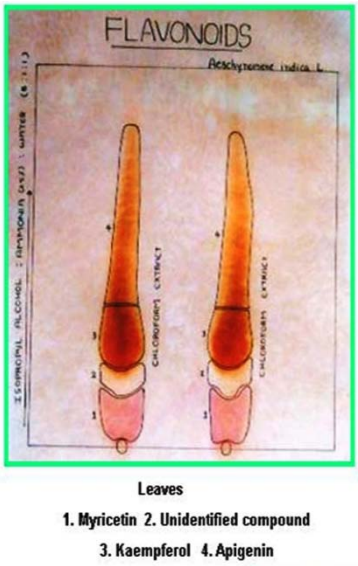
Solvent: Isopropyl Alcohol: Ammonia (25%): Water (8: 1: 1) v/v/v.

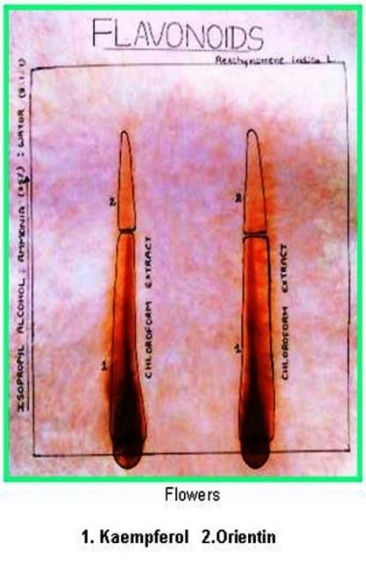
* Compound showing more Color; **Compound showing more Quantity

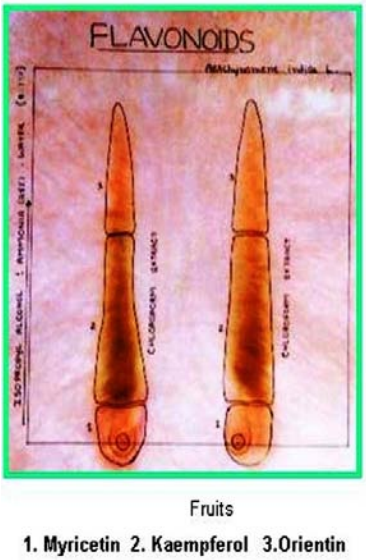
***Compound showing more Colour & Quantity.

PLATE 2:

Flavonoids







Qualitative Analysis of Anthocyanidins in Flowers: (Table-7) (Plate-3)

Flowers contain Delphinidin, Malvidin, and Peonidin, but delphinidin and malvidin are the main compounds.

Table 7: Anthocyanidins in Flowers

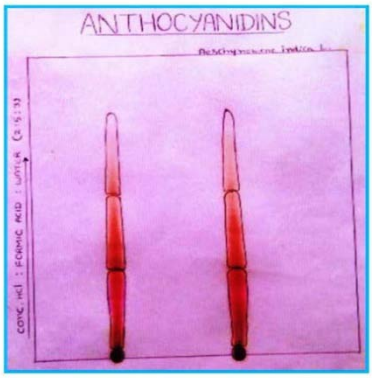
S. No.	Rf	U V Fluorescence		Visible Colour	Identified Compound
		Without NH_3	With NH_3		
1	0.15	Mauve	Blue green	Purple	Delphinidin*
2	0.4	Mauve	Blue green	Light purple	Malvidin**
3	0.65	Pink	Blue	Magenta	Peonidin

Solvent: Isopropyl Alcohol: Ammonia (25%): Water (8: 1: 1) v/v/v.

*Compound showing more Color ** Compound showing more Quantity.

PLATE 3

Anthocyanidins



Flowers

1. Delphidin 2. Malvidin 3. Peonidin

Antimicrobial activity (Table-8) (Plate-4)

Antibacterial activity of leaf extracts with Alcohol, Methanol, hot water and cold water on the selected four pathogens resulted as follows. *S.aureus* is most susceptible with Alcoholic extracts at 10mg-41mm activity. and *E.coli* is more resistant at with 29 mm with methanolic extracts .Other pathogens as *P. aeruginosa* and *E. coli* also shows more susceptible with alcohol extracts. When compared to other extracts Effective inhibition of the leaf extracts was observed highest Alcohol extracts only.

Table 8: Antibacterial Activity of Leaf Extracts 10 mg/well

S. No.	Organism	Cold Water	Hot Water	Methanol	Alcohol	Controls	
						A-10 mg	G-30 mg
1	*B. subtilis*	21±0.9	26±1.8	20±1.8	32±0.2	18±1.4	18±0.5
2	*S. aureus*	16±3.2	17±1.8	18±2.3	41±0.4	29±1.4	22±0.4
3	*P. aeruginosa*	20±1.8	14±1.8	20±1.4	35±0.6	25±1.4	19±1.6
4	*E. coli*	17±1.8	13±0.9	29±0.9	35±0.8	20±0.4	20±0.4

Values are mean Inhibition zone (mm) ± S.D of quadruplicates.

A= *Ampicillin*, G= *Gentamycin*

MIC (Table-9)

Leaf alcoholic extracts are more effective in showing MIC values at very low concentrations against all pathogens ranging between 0.46mg to 0.75 mg followed by Methanol, Cold Water and Hot Water an average values ranging from 0.53mg to 0.78 mg against all organisms. *B.subtilis* is showing very effective at 0.46mg followed by *P.aeruginosa* at 0.48mg with alcohol extracts.

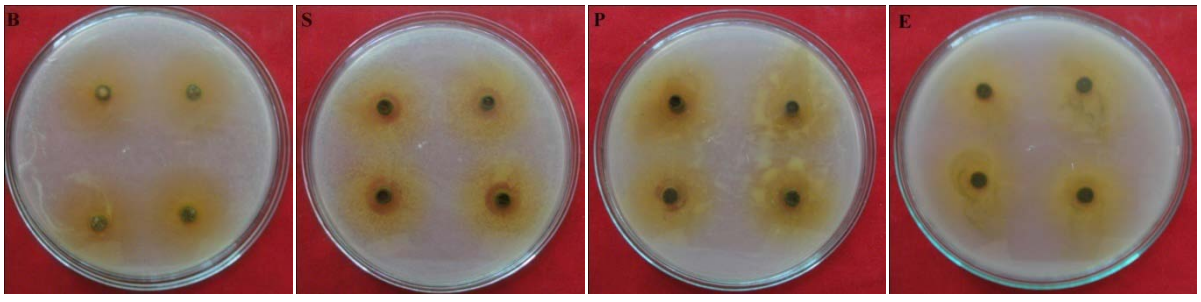
Table 9: MIC values for leaf extracts (in mg)

S. No.	Organism	Cold Water	Hot Water	Alcohol	Methanol
1	*B. subtilis*	0.53	0.64	0.46	0.72
2	*S. aureus*	0.61	0.70	0.70	0.78
3	*P. aeruginosa*	0.70	0.60	0.48	0.56
4	*E. coli*	0.54	0.78	0.75	0.78

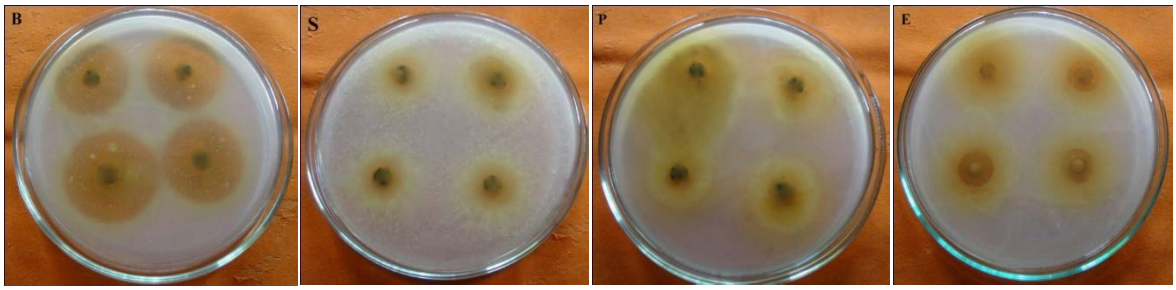
PLATE 4

Antibacterial Activity of the Leaf Extracts

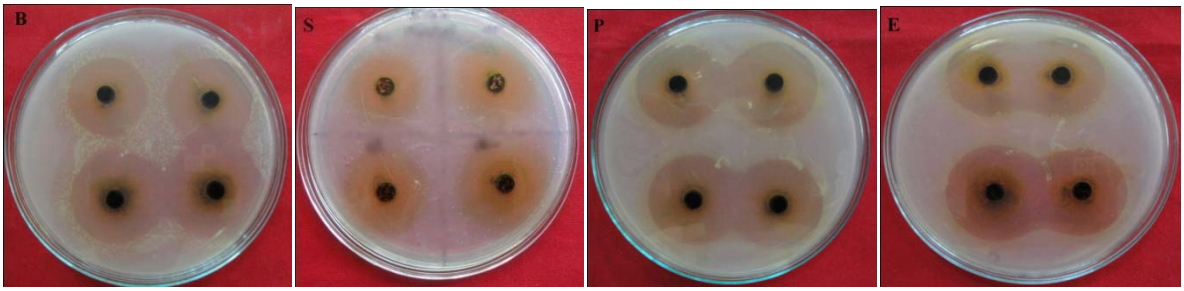
Cold water



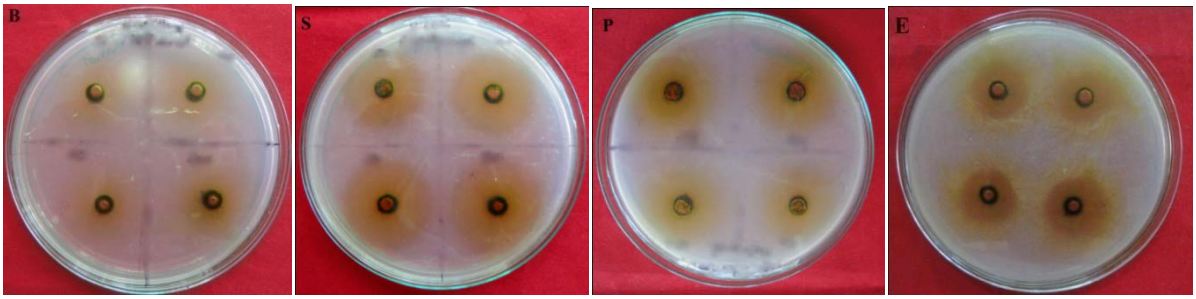
Hot Water



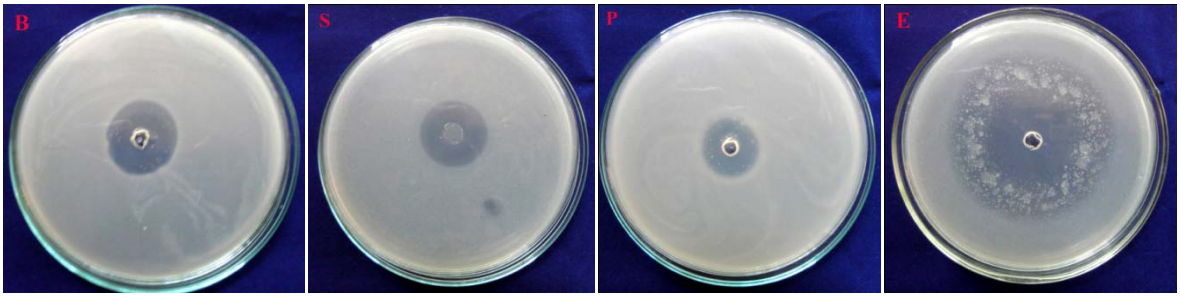
Alcohol



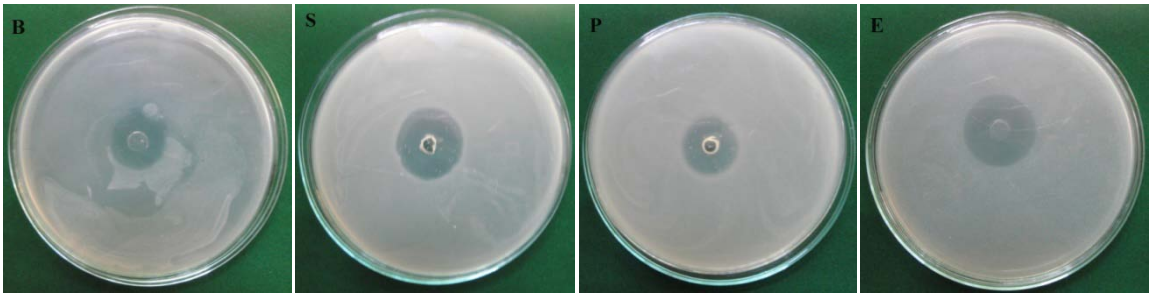
Methanol



Ampicillin



Gentamycin



B: *Bacillus subtilis* **S:** *Staphylococcus aureus* **P:** *Pseudomonas aeruginosa* **E:** *Escherichia coli*

DISCUSSION

Phenolic compounds like coumarins and melilotic acid are isolated from *Glyricidia sepium* through cell culture[20] P-Hydroxy benzoic acid, vanilic acid, trans- P-coumaric acid and some other compounds are isolated from *Rhynchosia cyanosperma* [21]. *Abrus precatorius, Cassia angustifolia, C.obtusifolia* leaves used against Jaundice[22]. *Sesbania grandiflora* leaf with Tannins and glycosides[23]. *S. sesban* leaf consists saponins glucoronide- Oleonolic acid[24]. *Tephrosia purpurea* whole plant, seeds of *Clitorea ternata*, roots of *Indigofera ternata, Pseuderthria viscid* roots, heart wood of *Pterocarpus santalinus*, roots of *Mucuma pruriens* and *Pisum sativum* are used against Leprosy [25]. From *Phaseolus, Vigna, Macroptilium, Strephostyles* and *Dysolobium* 35 compounds of flavonoids are screened out[26]. *Butea monosperma* leaves, seeds of *Dolichos biflorus* and *Desmodium motorium* leaves and bark of *P.marsupium* are used against diabetes[27] *S.bispinosa* seeds are emmeragogue, stimulant, astringent, heals chronic ulcers, removes small pox eruptions, acts against spleen diseases, antidiarrhoeal, anthelmintics, demulcent in hydrocele[4] *Dalbergia sissoo* leaves used against bronchial disorders like asthma and pulmonary infections[28]. *A.precatorius* leaves and roots contain many flavonoids, aminoacids, alkaloids and are used against cough, cold, pains, skin diseases, diuretic and against Jaundice. Seeds are purgative Aphrodisiac, Abortifacient. *C.ternata* leaves contain kaempferol, flowers with Delphinidine, Malvadin and kaempferol, white flowers with only kaempferol, seeds with flavonoids, flavones. Leaves are used in otalgia, hepatopathic and eruptions, seeds cathartic, used in Viseralgiasic. *Pongamia pinnata*, *Psoralea erycifolia, T. purpurea* leaves used against skin diseases[29]. Alkaloids from *A.precatorius* tannins, saponins and alkaloids from *Caesalpinia pulcherima* are reported[30].

Chlorogenic acid and kaempferol-3, 5-β-D-diglycoside are isolated from the leaves of *Indigofera hirsuta*[31]. Also reported presence of 9 Phenolic compounds mainly proto catechuic acid, chlorogenic acid, trans-p-coumaric acid, caffeic acid, p-hydroxy benzoic acid, coumarin and cinnamic acid, six Flavonoid compounds like rutin, quercetin, kaempferol, luteolin, apigenin and orientin[32]**;** shows effective antimicrobial activity, used as a dose against infant immunity[33]**.** Presence of proanthocyanidin in the seeds of eleven *Vigna* species contains delphinidin, kaempferol and cyanidin as the main flavonoids[34]**.** *C.ternata* with potential neutrachemicals having delphinidin and malvidin reduces upper respiratory infections and also various forms of cancer [35]**.**

Mucuma pruriens consist alkaloids like mucunine, mucunadine, prurine and prurienine [36]; leaves, roots, seeds are commonly used in the treatment of impotence, snake bite, diabetes, cancer and parkinsonism. Endocarp is non –toxic and is 2-3 times potent than leavodopa in controlling hyperprolactinemia[37], also used against motor symptoms of Parkinson's disease[38] *M. pruriens* leaves as remedy for diabetes, arthritis, dysentery and cardio vascular[39]; seeds contain 5-indolic compound especially tryptamine and hydroxy tryptamine[40]; also neuroprotective by significantly restoring dopamine and nor epinephrine levels in Parkinsonism[41]**.** *Pueraria tuberose* is an important medicinal plant used as aphrodisiac, leprosy, urinary blood discharge[42]. *P.tuberosa* in folk medicine root tuber is applied for blood purification and to improve sperm production; root powder controls tumor growth in stomach, and controls fertility levels of women[43]. *P.santalinus* aqueous and ethanolic stem bark extracts against hepatoprotective activity with the presence of alkaloids, phenols, saponins, glycosides, flavonoids, sterols and tannins[44]. *Pterodon emarginatus* seeds with phenolic and flavonoid constituents shows antioxidant activity[45].

T.purpurea contains many phenolic and flavonoid compounds and acts against bronchitis, boils and pimples, bleeding piles, dyspepsia, diarrhea, rheumatism and urinary bladder disorders. Uterine infections and also as abortifacient[46]. *Glycirrhiza* species are reviewed for their active compounds and biological activity [47].

Apigenin was isolated from *I.mysurensis*, *G.glabra;* and ethanolic extracts against diabetes[48]. Apigenin was isolated from *Trigonella scoparia*, chalcones and deoxy flavones from *Phaseolus aureus,* apigenin from *Glycine max* through cell cultures[49]. *G.uralensis* was tested against anti-tumor activity on human cell lines[50] also antibacterial against *S.mutants*[51]; root extracts acts as estrogenic on breast cancer cells[52], hexane and ethanolic extracts arrests human prostate and murien mammary cancer cells[53].A new acetylated isoflavone from *P.santalinus* acts against inflammations, mental aberrations, ulcers and in diabetes. Stem bark and leaf extracts acts as antibacterial[54]. Apigenin and luteolin from *Scutellaria barbata* acts against (MRSA) Methicillin resistant *Staphylococcus aureus*[55].

Anthocyanosides from berries are used in ophthalmology to improve vision and prevent diabetic retinopathy[56-57]**.** Anthocyanins and anthocyanin-rich extracts are with least adverse effects, showing clinical interest[58-59].Dimethoxy ether and glycoside of leucopelargonodin isolated from *Ficus benghalensis* bark showed significance hypolapidemic and serum insulin raising effects to that of control drug[60-63]**.** Anthocyanosides improve and normalize capillary filtration of albumen[64]**.** Anthocyanin extracts of *Vitis myrtillus* are reported vasorelaxation and ophthalmic activity[65-66]**.** Consumption of Pomegranate juice in diabetic patients resulted in antidiabetic state in their serum and the oxidative state of monocytes/ macrophages levels.[67] Flavonoids including anthocyanins associated with low prevalence of some diseases such as cancer and cardiovascular diseases[68]**.** Cyanidin 3-glucoside may ameliorate high fat diet-induced insulin resistance in mice[69]**.** Purified high doses of anthocyanin oligomers on nocturnal visual function and clinical symptoms on patients with asthenopia and refractive errors on both eyes reported 73.3% improved symptoms[70]**.** Several naturally extracted or synthetic anthocyanins prevent occurrence of cataracts or anti-glucoma activity[71-72]**.**

Bauhinia nitida and Cassia occidentalis leaf extracts proved their antioxidant and also anti- inflammatory activities due to the presence of flavonoid and Phenolic compounds[73]**.** *C.ternata* root methanolic extracts as anti-inflammatory, analgesic and antipyretic[74], *P.indicus* leaf, root and stem bark extracts as antimicrobial[75]. Methanolic root and bark extracts of *Peltophorum africanum*; bark extracts of *M.coriacea* and whole plant extracts of *Zorina minima* as antiviral and antibacterial[76]. Aqueous and alcoholic extracts of *Vigna radiata*, *Arachis hypogea* and *Canavalia gladiata* acts against *Staphylococcus* [77]. Isoflavones reported from *Atylosia* and *Cicer* species[78]. Leaf extracts of *Daniella oliver* as antidiarrhoel[79]. Antipneumonial activity of *Erythrina senegalensis*, *Piliostigma thanningii* and *Andera innermis* against Pneumonia[80]. *T.purpurea* seed aqueous extracts as antioxidants and antidiabetic[81]. *Bauhinia purpurea* stem ethanolic extracts against diabetic activity and androgenic property[82]**.** Leaves of *Boerhavia diffusa, Baphia nitida, Cassia occidentalis, Desmodium cinerea* used treatment if asthma in ivory coast. *D.cinerea* as one of the effective drug against asthma[83]**.** Alcoholic root extracts of *Piptadeniastrum africanum* as anti proliferative on human colonic cancer cells.[84] Leaf ethanolic extracts of *I.tinctoria* as anti-inflammatory[85].

Delphinidine is a major Anthocynadin possess antioxidant, anti-inflammatory and antiangiogentic properties and it is also suggested as a novel agent against prostate cancer (PCa).[86] Delphinidin and Cyanidin- 3- O-sambubiosides of *Hibiscus sabdariffa* reduces blood pressure in humans due to angiotensin converting enzyme (ACE) inhibitor activity with an IC 50 value of 84.5 and 68.4 g/ml[87]**.** Both cyanidin and delphinidin exerted cytotoxicity in primary and metastatic colorectal cancer cell lines[88]**.** Isolated Delphinidin from *Punica granatum* (DP) inhibited histone acetyltransferase activity (HAT) (which contributes rheumatoid arthritis on human synovial cell lines[89]**;** It also induces apoptosis of human prostate cancer PC3 cells *in vitro* and *in vivo* studies, and induces necrosis in hepatocellular carcinoma cells; also inhibits HCR2 and erk ½ signaling and suppresses growth of HCR2 over expressing and triple negative breast cancer lines[90].Dietary flavonoids play an important role in prevention of human cancers related to carcinogenesis a multistep process[91]**.** *H.sabdariffa* a popular jamican herbal medicine as antihypertensive and diuretic has been shown evidence against diuretic, nutriuretic and potassium-sparing effects[92].

Pterocarpus marsupium stem methanol extracts on *B.coagulens* and *E.coli* at 100 mg/ml concentrations shows effective inhibition to that of the control drug *Ciprofloxacin*[93]. *Saraca asoca* Phytochemical, qualitative analysis through HPTLC resulted tannins, flavonoids, carbohydrates, proteins, steroids in different extracts of flowers and

also identified gallic acid as active constituents both in flowers and leaves[94]. Comparative antibacterial studies of *Acacia nilotica* both *in-vitro* and *in-vivo* studies proved more efficient with callus methanol extracts than bark and leaf extracts with MIC values ranging from 1.9±0.02mg/ml[95]. *A. nilotica* young leaves and bark extracts yielded high quantities of tannins which are rich in phenolics are proved as good anti-oxidants through *in-vitro* models[96].

Aeschynomene sensitiva and *A. indica* are reported to possess insecticidal activity[97]; spermicidal[98-99]. From *A.indica* reynoutin and aeschynomate are isolated[100]. Roots of *A.mimosifolia* yielded neoflavonoids, mimosofolial and mimosifolenone[101]. *A.aspera* yielded highest number of secondary metabolites; leaves contain 11 Phenolic compounds mainly neo-chlorogenic acid, and homo-protocatacheuic acid, caffeic acid, phloroglucinol, trans-p-coumaric acid, cinnamic acid along with flavonoids like myricitin, apigenin and quercitin which are used to cure uric acid calculi and urinary troubles and against sperm count increase. Delphinidin and peonidin are also present in *A.indica* may exhibit their potential effects on the cardiovascular diseases, antidiabetic, antiglucoma and antihypertension also as anticancerous[102].

CONCLUSION

Ethno botanical uses and the presence of Phytoconstituent are in correlation with therapeutic activity of the other medicinal plants of Fabaceae. *A.indica* possess coumarins (*G. sepium*), p-hydroxy benzoic acid, vanilic acid, coumarins and trans-p-coumaric acid (*R.cyanosperma)* tannins and glycosides (*S.grandiflora*), saponins (*S.sesban*), flavonoids (*Phaseolus, Vigna, Macroptilium, Strephostyles* and *Dysolobium*), kaempferol (*I.hirsuta, C.ternata*), flavonoids, alkaloids (*A.precatorius*), alkaloids, phenols, saponins, glycosides, flavonoids, sterols and tannins (*P.santalinus, Caesalpinia pulcherima*), apigenin (*Indigofera mysurensis, G.glabra, G.max, S.barbata*).

Therapeutic activities of *A.indica* also correlated with that of *T.purpurea, C.ternata, C.ternata, P.viscida, P.santalinus, M.pruriens* and *P.sativum* against Leprosy. *S.bispinosa* and *M.pruriens* as a stimulant. *A.precatorius* seeds as purgative and aphrodisiac, spermicidal. The effective antibacterial and antifungal activities of *A.indica* as that of *P.indicus, P.africanum, M.coriaceae, Z.minima*. And also the effective inhibition against (MRSA) *Staphylococcus aureus* is very important to that of *V.radiata, A.hypogea, C.gladiata* in showing its potential therapeutic activities as mentioned above. And also the main Herbal and Folklore usages of the species against kidney stones may be due to the presence of phenols, flavonoids, anthocynadins, steroids, terpinoids, saponins, tannins and glycosides in toto mainly by the presence of isochlorogenic acid, hydroxy benzoic acid, caffeic acid, coumarins, myricitin, apigenin, kaempferol, delphinidin, cynanidin and peonidin. The collective activity of all phytoconstituents may be the reason in healing the kidney stones. Physicochemical studies like extractive values, ash contents, histochemical and fluorescence analysis also supports its purity and quality to assess the drug in its various formulations and also to measure the adulterants. Further the phenols, flavonoids and anthocyanidins which are present in A.indica may also act as antioxidants, anti-inflammatory, anti carcinogenic, anti estrogenic, antiviral against Herpes simplex virus Type I and Type II; skin protection and skin tumors (coumarins), cardiovascular, anteroscleroids, prostate cancer, pancreatic cancer, antileukaemic (myricetin, kaempferol), hepatoprotective (apigenin), antidiabetic, antihypertension and anticancerous (delphinidin, cyanidin and peonidin).

Hence *A.indica* may be recommended as one of the best herbal medicinal plant in healing the kidney stones, leprosy and as potential drug in enhancing sperm count. Studies on in vitro production of the significant secondary metabolites, isolation of pure compounds; and screening for its clinical biological activities to that of herbal uses are in need also *A.indica* has to conserve through In-vitro and In-vivo studies.

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